Keywords: Bronchopulmonary dysplasia, Low birth weight, Mortality, Morbidity, Nutrition supplementation

ABSTRACT
Vitamin A is involved in regulation of fetal lung growth. It protects the preterm lung from oxidative damage. It maintains the integrity of respiratory tract epithelium and helps in differentiation of epithelial cells. In addition, it is necessary for the formation of photosensitive visual pigment in the retina. Preterm neonates, especially very low birth weight (VLBW) have lower concentrations of plasma retinol binding protein (RBP) and plasma vitamin A concentrations than term infants. The plasma vitamin A concentrations in preterm infants with bronchopulmonary dysplasia (BPD) are still lower. Inadequate provision and delivery of vitamin A during postnatal period exacerbates this deficiency and makes these infants prone to multisystem diseases. We review here the current evidence regarding vitamin A supplementation in VLBW and extremely low birth weight (ELBW) infants, and hypothesize important research priorities in this regard.

INTRODUCTION
Vitamin A maintains the integrity of epithelial cells of the respiratory tract and is an essential element for normal lung growth [1]. It is also necessary for the formation of the photosensitive visual pigments in the retina [2]. Other important role of vitamin-A include its action as antioxidant agent and boosting the immune system [3,4]. Vitamin A deficiency has been shown to produce histopathological changes in the respiratory tract epithelium; which reverses after therapeutic vitamin A supplementation [5].

BPD occurs in premature neonates requiring prolonged mechanical ventilation and oxygen therapy for acute respiratory distress [6,7]. The overall incidence of BPD, has not changed over the past decade [8,9]. The disease increases the cost of survival particularly in VLBW neonates [10]. Clinical trials suggest that vitamin A deficiency and alterations in vitamin A metabolism increases the risk of BPD in premature VLBW neonates [11]. Furthermore, it has been hypothesized that treatment of the neonates prone to develop BPD with therapeutic doses of vitamin A can reduce the incidence of BPD and several studies have been undertaken to assess this issue [1,12,13].

We briefly review the metabolism of vitamin A, and explore the evidence to determine the effect of vitamin A supplementation in premature VLBW and extremely low birth weight (ELBW) neonates. We also put forward few unanswered questions on vitamin A supplementation in this group of neonates which might give a direction for future studies in this field.

VITAMIN A: METABOLISM AND FUNCTIONS
Vitamin A refers to a group of compounds which includes retinol, retinaldehyde and retinoic acid [14]. Retinol may be obtained directly from foods of animal origin or be formed in the body from metabolism of β-carotene. Absorption of dietary retinyl esters is complex, involving hydrolysis and complex formation with bile acids in the gut lumen before uptake by enterocytes. Metabolism of vitamin A within these cells and subsequent transfer into the lymphatic system is by a specific carrier protein (cellular retinol binding protein [RBP] type 2). The availability of RBP type 2 is limited in preterm infant [15]. After absorption, retinol is bound to RBP in the liver and transported in plasma as the retinol-RBP complex, bound in a 1:1 ratio with transthyretin. Circulating retinol is delivered to target tissues via a specific membrane receptor and is oxidized within the cell to its active metabolite i.e. retinoic acid [Table/Fig-1].

Vitamin A after entering inside the respective cells performs various functions including formation of rhodopsin pigment for vision and maintenance of the epithelial cell integrity of respiratory, cardio-vascular...
and other organ systems [16,17]. It’s role has been proposed in the constriction of patent ductus arteriosus in animal models [18]. In addition, it may be involved in the development of an appropriate balance of key regulatory immune cells and specific mucosal antibodies in the gut, which is essential in the initial periods of life [4].

**ETIOLOGY OF VITAMIN A DEFICIENCY IN VLBW NEONATES**

Transfer of vitamin A to the fetus occurs throughout the pregnancy, and the accretion is maximum in the last trimester. The placental transfer is insufficient if there is severe maternal deficiency of vitamin A. Preterm neonates (especially those born before 32 weeks) are born with inadequate body stores of vitamin A [19]. These neonates have low plasma concentrations of both plasma retinol (defined as <0.7 mmol/L) [11,20], and retinol binding protein (RBP) at birth compared with those born at term. Even relatively healthy preterm neonates have low plasma retinol concentrations (<0.7 mmol/L); in ELBW group this value is still lower (less than <0.35 mmol/L at day 28 of life) [12,21,22]. This deficiency persists throughout the first year of life [23]. The plasma retinol concentration is closely correlated with that of RBP and reflects the hepatic stores [22]. The renal loss of retinol from decreases over time as the period of gestation increases or maturity occurs.

**OUTCOMES OF VITAMIN A DEFICIENCY IN VLBW NEONATES**

1. **Pulmonary diseases**

Vitamin A is required for the lung development in utero and thereafter. The four stages of lung development including the alveolar septation would be deficient/poorly functional if there is vitamin A deficiency. Vitamin-A deficient normal infants have shown pathological changes in the lungs similar to that seen in BPD [1]. Preterm neonates with coexistent BPD, often manifest clinical, biochemical, and histopathological evidence of vitamin A deficiency, unlike those with no lung disease. This is due to the fact that, hepatic stores of vitamin A get reduced in preterm neonates developing BPD. BPD is ascribed to increased production of cytotoxic oxygen free radicals, which can cause lung injury [6,24]. Premature neonates have low levels of antioxidants, thus increasing their vulnerability to oxygen toxicity. The pathophysiological consequences resulting from vitamin A deficiency are similar to BPD and include - loss of normal secretions of goblet cells, loss of normal

![Vitamin A metabolism in the body](image-url)
water homeostasis across tracheobronchial epithelium, loss of mucociliary transport with resultant predisposition to airway infection, narrowing of lumen and loss of distensibility of airways with resultant increase in airway resistance [25], thus contributing to increase in work of breathing. The histopathologic changes of vitamin A deficiency and associated pathophysiologic consequences are reversible with restoration of normal vitamin A status. So, a possible resolution or reversal of BPD changes with vitamin A supplementation can be proposed. This pathophysiological hypothesis was the basis for different trials studying the role of vitamin A in prevention of BPD in this group of neonates.

2. Eye disease
Vitamin A in its physiological (retinol) form helps to protect the photoreceptor cell membrane from the oxidative stress related damage. As a result, the rhodopsin present in the photoreceptors during developmental phase gets less susceptible to the fluctuating oxygen concentration (hypoxia-hyperoxia cycle)/other obnoxious agents associated with the onset and progression of retinopathy of prematurity (ROP). A recent Cochrane systematic review showed a non-significant reduction in the ROP in vitamin A supplemented neonates [26]. But the largest trial included in this review did not report about this outcome in preterm ELBW neonates [12]. In the study by Ambavalan N et al., the rate of threshold ROP was nil in a group of ELBW neonates who received intramuscular vitamin A supplementation (10,000 IU thrice weekly) compared to 16% in the group that received half the dose [25]. Another study by Mactier H et al., used the above dose schedule of vitamin A, but was not adequately powered to look at the incidence of ROP in a group of VLBW neonates [27].

3. Patent ductus arteriosus (PDA)
Animal models have shown that antenatal administration of vitamin A accelerates the contraction and closure of PDA. Post-natal administration of vitamin A (2,000-3,000 IU/kg, intramuscularly, thrice weekly) has not shown to affect the closure rate of PDA in ventilated VLBW neonates [16]. But, the vitamin A dose used in this study was less than the dose shown to improve the respiratory outcome subsequently.

4. Neonatal sepsis
Though vitamin A is an immunostimulator, a study has shown a trend towards non-significant reduction in the rate of neonatal sepsis (culture positive, hospital-acquired) in vitamin A supplemented neonates [12]. However, another recent study did not find any benefit in ELBW neonates [28].

5. Intra-ventricular haemorrhage (IVH)
There are speculations that vitamin A supplementation may have a role in the reduction of incidence and grade-severity of IVH. This was because, a study showed an increased incidence and severity of IVH in neonates having low hepatic vitamin A store. However, another study trying to validate this finding by supplementing vitamin A in the postnatal period in a group of ELBW neonates, did not find any benefit for the incidence of severe IVH [12,29].

6. Periventricular leukomalacia (PVL)
Though unexplained, vitamin A supplemented neonates have shown a trend towards non-significant reduction in the rate of Periventricular leukomalacia at one-month age [12].

VITAMIN A TOXICITY
Excess supplementation of vitamin A however is potentially harmful [29] and can cause transient acute side effects, such as bulging fontanelles, vomiting, diarrhoea, loss of appetite and irritability. Most common is the bulging of the fontanelle, but it is a benign condition and not associated with acute or long-term neurodevelopmental effects [30].

EVIDENCE FOR VITAMIN-A SUPPLEMENTATION IN NEONATES
There are many randomized trials and systematic reviews on the supplementation of Vitamin A and clinical/laboratory outcomes in preterm VLBW neonates.

The Cochrane review included nine randomized controlled trials (RCTs) comparing vitamin A supplementation with a control (placebo or no supplementation) or other dosage regimens in VLBW neonates (birth weight ≤ 1500 g or < 32 weeks’ gestation). Out of these, 8 compared vitamin A supplementation with a control (n = 1291), and 1 compared different regimens of vitamin A (n = 120) [26]. The results showed that, vitamin A compared to the control group is beneficial in reducing death or oxygen requirement at one month of age [relative risk (RR) 0.93; 95% confidence interval (CI), [0.88 to 0.99] with the number needed to benefit (NNTB) being 20 (95% CI, 10 to 100) and oxygen requirement at 36 weeks postmenstrual age (RR, 0.87, 95% CI, 0.77 to 0.98) NNTB 13 (95% CI, 7 to 100). A trend towards a reduction in death or oxygen requirement at 36 weeks postmenstrual age was also noted (RR, 0.91; 95% CI, 0.82 to 1.00). Neurodevelopmental assessment of 88% of surviving infants in the largest trial [30], showed no differences between the groups at 18 to 22 months of age, corrected for prematurity. The different dosage vitamin A regimens showed similar results in terms of BPD or death.

In a recent study by Gadhes MM et al., early iNO therapy and vitamin A supplementation was found to reduce the risk of BPD in premature neonates (birth weight of 750-999 g) compared with iNO therapy alone. The combined therapy was found to improve the Bayley Scales of Infant Development II Mental and Psychomotor Developmental Index scores at one year compared with infants treated solely with iNO therapy [28]. However, in a recent largest retrospective study (n = 3,011) by Tolia VN et al., trying to find out the real role of national shortage of vitamin A on the rate of death or BPD in ELBW neonates found no effect of the former on the later [31]. This later study
finding has important implications regarding the role of vitamin A supplementation in ELBW neonates at present. It indicates that, other strategies like gentle ventilation or non-invasive ventilation and targeted oxygen saturation may replace the use of vitamin A in management of ELBW neonates in near future.

**ROUTE OF SUPPLEMENTATION**

Vitamin A can be given either enterally, intramuscularly, or intravenously. In term neonates, vitamin A is well absorbed enterally. In VLBW neonates, vitamin A given orally in conjunction with early feeds can achieve comparable plasma concentrations of retinol to vitamin A given intramuscularly if large oral doses are given [32]. In ELBW neonates, however, even very large enteral doses of vitamin A from birth neither significantly increase plasma concentrations nor improve outcomes, possibly due to immature gastrointestinal function [13,33]. There are also problems with the parenteral intravenous administration of vitamin A, as it gets inactivated when it comes into contact with light and oxygen [34,35]. Furthermore, when added to glucose–amino acid solutions, it adheres to the standard plastic tubing resulting in 50 to 80% decrease in the effective delivery of the drug to the neonate [34-36]. If added to intravenous lipids (instead of glucose–amino acid solutions) the effective delivery is increased to approximately 77 to 86% [37]. However, this method is limited only to neonates receiving total parenteral nutrition. Considering all these, the preferred route for vitamin A administration in ELBW neonates would

<table>
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<th>Author, year</th>
<th>Method</th>
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| Tyson et al., 1999 [12] | Multicenter RCT | • Intervention = 405; Control = 402  
• Birth weight = 401-1000 gm  
• Inclusion criteria - Requirement of supplemental O2 or mechanical ventilation at 24 h | IM vitamin-A, 5000 IU three times per week for 4 weeks | • Reduced BPD or mortality at 36 weeks RR (95% CI) = 0.89 (0.8-0.99)  
• NNT 14-15 Sepsis, IVH, PVL; Non significant trend towards reduction. | Used IM route  
No adverse events reported |
• Birth weight = 700-1100 gm  
• Inclusion criteria - requirement of supplemental O2 or mechanical ventilation at 96 h | IM vitamin-A, 2000 IU alternate days from day 4 to day 28 (14 doses) | • No difference between the groups on  
| | | | | | Use IM route, intervention started after 96 hours of age |
| Ravishankar et al., 2003 [16] | RCT | • Intervention = 22; Control = 18  
• Birth weight = 500-1500 gm  
• Inclusion criteria - infants with indwelling umbilical line. | IM vitamin-A, 1500 – 3000 IU on days 1, 3, 7 | • No difference between the groups for PDA closure  
• No difference between the groups for death, BPD, IVH. | Used IM route, used few doses of vitamin A (3 doses) |
| Shenai et al., 1987 [20] | RCT | • Intervention = 20; Control = 20  
• Birth weight = 700-1300 gm  
• Inclusion criteria - requirement of supplemental O2 or mechanical ventilation at 72 h | IM vitamin-A, 2000 IU on alternate day from day 4 to day 28 (total 14 doses) | • Decrease in the incidence of BPD (I = 9/20, C = 17/20, p <0.008).  
• Reduction in the need for mechanical ventilation (p < 0.029). | Used IM route |
| Wardle et al., 2001[13] | RCT, Multicenter | • Intervention = 77; Control =77  
• Birth weight <1000 g  
• Inclusion criteria - infants <24 h | Oral vitamin A 5000 IU per kg/day until 28 days of life | No difference in BPD | The dose might have been insufficient |

**Table/Fig-3**: Vitamin A dose adequacy  
CPAP = Continuous positive airway pressure, PMA = Postmenstrual age
be intramuscular rather than oral or intravenous route. Presently, a multicenter, double-blind RCT comparing postnatal high-dose oral vitamin A supplementation (5,000 IU vitamin A/kg/day vs. placebo) for 28 days in ELBW neonates is going on (NeoVitaA trial). With the publication of the results of this trial, the picture regarding oral route of supplementation will get clear [37]. These have been summarized in the tables [Table/Fig-2-4].

ISSUES THAT ARE STILL NOT ANSWERED

Though the Cochrane review generalizes its findings (beneficial effect) to preterm VLBW neonates, this might not be totally true, as the largest study by Tyson JE et al., [12], including 807 ELBW neonates found the beneficial effects in the ELBW infants only. Another small study by Shenai JP et al., [20], included 40 infants with birth weights of 700 to 1330 g, and found the beneficial effect of vitamin A supplementation. On the other hand, other studies have failed to demonstrate any beneficial effect of vitamin A supplementation [11,13]. The Shenai study was published in 1987 i.e the pre-surfactant era and the era of invasive ventilation. But currently with more use of surfactant and gentler modes of ventilation or non-invasive ventilations (e.g., CPAP), the vitamin A therapy may not really work. This is important for the evidence based clinical practice, as one can not recommend vitamin A supplementation for prevention of BPD or other outcomes in preterm neonates with birth weight >1000 g to <1500 g. This is an area for future research and we need more trials before any recommendation can be made regarding vitamin A supplementation in this group of neonates (or VLBW infants as a whole). Efforts should also be made to stratify the effect of vitamin A supplementation in preterm intrauterine growth restricted (small for gestational age) neonates. At the same time the research needs to be built on stable intravenous preparations which can resist degradation and can be safely given in fluids to these vulnerable neonates.

CONCLUSION

Vitamin A supplementation may reduce the morbidities (mostly respiratory) in ELBW neonates, particularly those requiring prolonged ventilation or supplemental oxygen. More good quality trials with larger sample sizes are needed on preterm VLBW neonates (including subgroup with birth weight >1000g to <1500g) along with the study of other outcomes (ROP, IVH, PDA, PVL, neuro-developmental outcomes) and alternate routes of administration (intramuscular vs. intravenous vs. oral), before any firm conclusion can be drawn.

REFERENCES


